

Chromosome 3, 69cM

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BACKGROUND: Dr. Y. Ji, University of Florida, indicated that there was an introgression associated with Ty4 gene on chromosome 3 near 76-85 cM. Originally, Ty4 was thought to be on chromosome 6 between 40-80 cM. Our UW-team scanned this region at 5-cM intervals and did not find any evidence for an introgression in Gc171, which gives the SCAR marker for Ty4. With this new information and the information from Dr. Ji, our group starting scanning chr. 3 from 66 - 85 cM to check for an introgression. Begomovirus resistant inbred used was G70, which was a selection from Gc171 by a susceptible hybrid. The susceptible germplasm was HUJ-VF (lab code, W168, an inbred from Hebrew University of Jerusalem, F. Vidavski and H. Czosnek) and M82.

Primers

P3-69F3: AATTGGCCAAATCATCATCTTTCTACCG

P3-69R1: AAGAGAGTGGTCAATGTCATCC

Annealing temperature 53 °C

Partial Sequence of G70 (resistant inbred), p3-69 F3/R1

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1      GTATCAGTAT CAGTAAATCT TAAGATTTAA TACTCTATTT TATTGTTTTT TAGTATGGAG
61     TCTCAAAAT TAAAATGGAA AGAAAAGCC CCATTTTTTG CTTTCTTAT TTGGGAGGAG
121    TGGGAGGTGG AGTCAGGATT TGAAGCTTAT TATGTGTTTC GAGCTATGTT GTTCTCGCTC
181    TCCCAAAATA CGCTATTTTT GGAGTATCCA ACACGTACCT GTTCATATTT TTGTGAAGTT
241    TGAGCATCAT GAGTTTCAAG TGGATTTTAA GGATGAATAG TAAATACAG GGTTTAAACC
301    AAAGCTAATG AATTCGTTTT TACTAAGGAA GGGATATTTA CATACCCAAG ATTACATTGA
361    TGTTAAATTT TAACCATTAT GTCCTTGCAC TTTCCAGTGG CTTTGTGAAA TTTTGAGATT
421    CATGGCTAAG AATGATCAGA AACAGTACAG ATAAGTTTTG TAGATGTTTG CCTTTTATGA
481    AACTTCTCTG TAATTTAGGC ATTGTCCTTG ACATGCTCG GTTCTTAAAT TAGAGATATT
541    TAATGTGACT TTTGAACTAC TATGCTATTT GCATCGTCGT CTATTATAGA CATTGATTGA
601    GTGGTTTTGA ATATGCTTTT GGCAAATAAT TTCTTGATC TTCTGAAATC ATGTTGTATT
661    TACAGGTAGA GGAGATAGGA TGGGAGCACC TTGTTAGATT TGGGAAAGAC CTAAGACTTC
721    TCAGCTTTCG GATCAAGTAT GGCCTCTTTT ATCTCTTAAAC GTTATGCTTG GGTAAAATTT
781    AGGAAGAGGA GGTGTTGGTT AAATGAAAGA AGAGGTATTA GATTGTTCCC TTGTTTGTTA
841    GGAAGGAATT ATAAGAGGAG GGACTGTAGT GGAGGACTGT AACTTCTGA ATTGCAGCTA
901    GTGGAAGACT GTGATTTGCC TTACCGGAAA ATAATCTCTC AAGCTTTTTT CGTGTGTTAG
961    GGACAAGAAG GGAAGAGTGC ACATTCGCA GATACTTTG GATGGAACAT ATCCTAACCA
1021   TCCGCCTTCA TTATCAGCGG TTGGCATCCT TCAAATTTGA GATACTAGAA CCTTGCATGA
1081   AGTCTTTTAG CAATGTCCTA ATTTACTGAC TATTCACCAG GATGTCCTT ATCTCTCAA
1141   TTTGAAATGG TCAATTAAC CAAGACTGAA AGATGTTATC CAACAGTTTC AGAAGGTAAT
1201   TGTGTATTCT TTTTATCATG TTGCAGCTCT CCCCTCGTTT GCATTTGCAT TGTAACCTAT
1261   GATTTACTAC TGATTATTC ATCCACCAAT GATATATGTG TAGTTTATCA ACATTTGTGA
1321   ATTGATGATT TCTGCTATAA TTACCATATC TTTTCTTAA TGGATCCAGC ATCTGAGAAG
1381   CTC
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Comparison of G70 (bottom sequence) with W168 (top sequence)


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961  GGACAAGAAGGGAAGAGTGCACATTCTGCAGATAACTTTGGATGGAACATATCCTAACCA
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
961  GGACAAGAAGGGAAGAGTGCACATTCTGCAGATAACTTTGGATGGAACATATCCTAACCA

1021 TCCGCCTTCATTATCAGCGGTTGGCATCCTTCAAATTTGAGATACTAGAACCTTGCATGA
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
1021 TCCGCCTTCATTATCAGCGGTTGGCATCCTTCAAATTTGAGATACTAGAACCTTGCATGA

1081 AGTCTTTAGGCAATGTCCTAATTTACTGACTATTCCCCAGGATGTGCCTTATCTCTTCAA
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
1081 AGTCTTTAGGCAATGTCCTAATTTACTGACTATTCCCCAGGATGTGCCTTATCTCTTCAA

1141 TTTGAAATGGTCAATTAACCTCAAGACTGAAAGATGTTATCCAACAGTTTCAGAAGGTAAT
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
1141 TTTGAAATGGTCAATTAACCTCAAGACTGAAAGATGTTATCCAACAGTTTCAGAAGGTAAT

1201 TGTGTATTCTTTTTATCATGTTGCAGCTCTCCCCTCGTTTGCATTTGCATTGTAACCTTAT
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
1201 TGTGTATTCTTTTTATCATGTTGCAGCTCTCCCCTCGTTTGCATTTGCATTGTAACCTTAT

1261 GATTTACTACTGATTATTTTCATCCACCAATGATATATGTGTAGTTTATCAACATTTGTGA
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
1261 GATTTACTACTGATTATTTTCATCCACCAATGATATATGTGTAGTTTATCAACATTTGTGA

1321 ATTGTGATTTTCTGCTATAAATTACCATATCTTTTTCTTAATGGATCCAGCATCT
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
1321 ATTGTGATTTTCTGCTATAAATTACCATATCTTTTTCTTAATGGATCCAGCATCTGAGAAG

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Comparison of M82(top sequence) with G70(Bottom Sequence)

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1    GTATCAGTATCAGTAAATCTTAAGATTTAATACTCTATTTTATTGTTTTTTAGNANGGAG
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
1    GTATCAGTATCAGTAAATCTTAAGATTTAATACTCTATTTTATTGTTTTTTAGTATGGAG

61   TCTCAAAATTTAAAATGGAAAGAAAAGCCCCATTTTTTGCTTTTCTTATTTGGGAGGAG
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
61   TCTCAAAATTTAAAATGGAAAGAAAAGCCCCATTTTTTGCTTTTCTTATTTGGGAGGAG

121  TGGGAGGTGGAGTCAGGATTTGAAGCTTATTATGTGTTCCGGAGCTATGTTGTTCTCGCTC
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
121  TGGGAGGTGGAGTCAGGATTTGAAGCTTATTATGTGTTCCGGAGCTATGTTGTTCTCGCTC

181  TCCCAAAATACGCTATTTTTGGAGTATCCAACACGTACCTGTTTCATATTTTTGTGAAGTT
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
181  TCCCAAAATACGCTATTTTTGGAGTATCCAACACGTACCTGTTTCATATTTTTGTGAAGTT

241  TGAGCATCATGAGTTTCAAGTGGATTTAAGGATGAATAGTTAAATACAGGGTTTAAACC
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
241  TGAGCATCATGAGTTTCAAGTGGATTTAAGGATGAATAGTTAAATACAGGGTTTAAACC

301  AAAGCTAATGAATTCGTTTTTACTAAGGAAGGGATATTTACATACCCAAGATTACATTGA
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
301  AAAGCTAATGAATTCGTTTTTACTAAGGAAGGGATATTTACATACCCAAGATTACATTGA

361  TGTAAATTTTAAACCATTATGTCCTTGCACTTTCCAGTGGCTTTGTGAAATTTGAGATT
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
361  TGTAAATTTTAAACCATTATGTCCTTGCACTTTCCAGTGGCTTTGTGAAATTTGAGATT

421  CATGGCTAAGAATGATCAGAAACAGTACAGATAAGTTTTGTAGATGTTGCCTTTTATGA
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
421  CATGGCTAAGAATGATCAGAAACAGTACAGATAAGTTTTGTAGATGTTGCCTTTTATGA

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Blast Search

Matched nothing on NCBI database greater than 90% full sequence on March 2, 2009. Matched with greater than 90% to the following from SOL genomics database:

Database: Tomato Combined Clone-end Sequences (BACs and Fosmids)

>[LE HBa0158C16 SP6 102654](#)

Length = 557

Score = 997 bits (503), Expect = 0.0

Identities = 548/559 (98%), Gaps = 3/559 (0%), Frame = +1 / +1

```
Query: 160 ggagctatggttctcgcctctccaaaatacgcctatTTTTGGAGTATCCAACACGTACC 219
          |||
Sbjct: 1   ggagctatggttctcgcctctccaaa-tacgcctatTTTTGGAGTATCCAACACCTTTT 59
```

```
Query: 220 -tgttcatatTTTTGTGAAGTTTGAGCATCATGAGTTTCAAGTGGATTTAAGGATGAAT 278
          ||
Sbjct: 60 atggtc-tatTTTTGTGAAGTTTGAGCATCATGAGTCCTAAGTGGATTTAAGGATGAAT 118
```

```
Query: 279 agttaaatacagggtttaaaccaaagctaataatcgTTTTACTAAGGAAGGGATATT 338
          |||
Sbjct: 119 agttaaatacagggtttaaaccaaagctaataatcgTTTTACTAAGGAAGGGATATT 178
```

```
Query: 339 tacataccaagattacattgatgtaaattttaaccattatgtccttgcactttccagt 398
          |||
Sbjct: 179 tacataccaagattacattgatgtaaattttaaccattatgtccttgcactttccagt 238
```

```
Query: 399 ggctttgtgaaatTTTGAGATTCATGGCTAAGAAATGATCAGAAACAGTACAGATAAGTTT 458
          |||
Sbjct: 239 ggctttgtgaaatTTTGAGATTCATGGCTAAGAAATGATCAGAAACAGTACAGATAAGTTT 298
```

```
Query: 459 tgtagatgTTTGCCTTTATGAACTTCTCTGTAATTTAGGCATTGTGCTTGACATTGCT 518
          |||
Sbjct: 299 tgtagatgTTTGCCTTTATGAACTTCTCTGTAATTTAGGCATTGTGCTTGACATTGCT 358
```

```
Query: 519 cggTTTCTAAATTAGAGATATTTAATGTGACTTTGAACTACTATGCTATTTGCATCGTC 578
          |||
Sbjct: 359 cggTTTCTAAATTAGAGATATTTAATGTGACTTTGAACTACTATGCTATTTGCATCGTC 418
```

```
Query: 579 gtctattatagacattgattgagtggtTTTGAATATGCTTTTGGCAAATAATTTCTTGGA 638
          |||
Sbjct: 419 gtctattatagacattgattgagtggtTTTGAATATGCTTTTGGCAAATAATTTCTTGGA 478
```

```
Query: 639 tcttctgaaatcatgTTGTATTTACAGGTAGAGGATAGGATGGGAGCACCTTGTTAGA 698
          |||
Sbjct: 479 tcttctgaaatcatgTTGTATTTACAGGTAGAGGATAGGATGGGAGCACCTTGTTAGA 538
```

```
Query: 699 tttgggaaagacctaagac 717
```

```
          ||||||||||||||||
Sbjct: 539 tttgggaaagacctaagac 557
```

>[LE HBa0025G10 T7 135848](#)

Length = 608

Score = 270 bits (136), Expect = 2e-70
Identities = 145/148 (97%), Frame = +1 / -1

```
Query: 1  g t a t c a g t a t c a g t a a a t c t t a a g a t t t a a t a c t c t a t t t t a t t g t t t t t t a g t a t g g a g 60
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 161 g t a t c a g t a t c a g t a a a t c t t a a g a t t t a a t a c t c t a t t t t a t t g t t t t t t a g t a t g g a g 102
```

```
Query: 61  t c t c a a a a t t t a a a a t g g a a g a a a a g c c c c a t t t t t t g c t t t t c t t a t t t g g g a g g a g 120
          |||||||||||||||||| || |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 101 t c t c a a a a t t t a a a a g g g a a g a a a a g c c c c a t t t t t t g c t t t t c t t a t t t g g g a g g a g 42
```

```
Query: 121 t g g g a g g t g g a g t c a g g a t t t g a a g c t t 148
          |||||||||||||||||||||||||||||||||||||||
Sbjct: 41  g g g g a g g t g g a g t c a g g a t t t g a a g c t t 14
```

Summary

The sequence data for G70 was very good data. When a comparison was done between G70 (resistant inbred) and either susceptible plant (M82 or W168), the results showed no differences in over 1300 bp. The above comparisons show the comparison data. The results of this experiment indicate that there is no introgression at 69 cM.